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Dorsal Pancreas Agenesis in N-Cadherin-Deficient Mice

Farzad Esni,* Bengt R. Johansson,† Glenn L. Radice,‡ and Henrik Semb*,[1]

**Department of Medical Biochemistry, Box 440, Göteborg University, S-405 30 Göteborg, Sweden; †Department of Anatomy and Cell Biology, Göteborg University, S-405 30 Göteborg, Sweden; and ‡Department of Obstetrics and Gynecology, University of Pennsylvania School of Medicine, 1355 Biomedical Research Building II/III, 421 Curie Blvd., Philadelphia, Pennsylvania 19104*

Members of the cadherin family of cell adhesion molecules are thought to be crucial regulators of tissue patterning and organogenesis. During pancreatic ontogeny N-cadherin is initially expressed in the pancreatic mesenchyme and later in pancreatic endoderm. Analysis of N-cadherin-deficient mice revealed that these mice suffer from selective agenesis of the dorsal pancreas. Further analysis demonstrated that the mechanism for the lack of a dorsal pancreas involves an essential function of N-cadherin as a survival factor in the dorsal pancreatic mesenchyme.

Key Words: **N-cadherin; pancreas; apoptosis; mesenchyme; endoderm.**

INTRODUCTION

It has been hypothesized that folding of epithelial sheets, such as budding of endodermal organs from the primitive gut, involves changes in cell polarity, cell migration, cell adhesion, and cell shape. The molecular mechanisms for these cellular processes are thought to encompass changes in the activity of cell adhesion molecules (CAMs) and dynamic rearrangements of the actin cytoskeleton. These epithelial cell behaviors are, apparently, under strict regulation by signals from neighboring mesenchymal cells. For example, during pancreatic ontogeny, growth and differentiation of committed pancreatic epithelial cells require soluble factors from mesenchymal cells in close vicinity (Golosow and Grobstein, 1962; Le Bras *et al.*, 1998; Miralles *et al.*, 1999). The fact that signaling by the mesenchyme in many tissues and organs (e.g., the intestine, skin, tooth, and kidney) correlates with condensation of mesenchymal cells suggests that cell adhesion may play a functional role in mesenchyme-to-epithelium signaling.

One of the most important and ubiquitous types of adhesive interactions required for the formation and maintenance of tissues is that mediated by the cadherin family of Ca^{2+}-dependent homophilic CAMs (Geiger and Ayalon, 1992; Steinberg and McNutt, 1999; Vleminckx and Kemler, 1999). The classic cadherins are defined by a highly conserved cytoplasmic domain that associates with a family of cytoplasmic proteins called catenins, including α-catenin, β-catenin, plakoglobin, and p120, which mediate the linkage to the actin cytoskeleton (Geiger and Ayalon, 1992; Nagafuchi and Takeichi, 1989; Ozawa *et al.*, 1989). Important for the full activity of cadherins is their clustering in cell–cell contact regions called adherens junctions. These subcellular structures act as important cell–cell signaling centers within cells and cadherins are of crucial importance for their establishment and maintenance (Barth *et al.*, 1997; Fagotto and Gumbiner, 1996; Vleminckx and Kemler, 1999).

N-cadherin has been shown to be involved in adhesive and signaling events during somitogenesis, skeletal myogenesis, bone formation, chondrogenesis, and patterning and segmentation of the brain (Duband *et al.*, 1987; Haas and Tuan, 1999; Larue *et al.*, 1996; Matsunami and Takeichi, 1995; Mege *et al.*, 1992; Oberlender and Tuan, 1994; Redfield *et al.*, 1997). Furthermore, N-cadherin-deficient mice die around 10 days postcoitum (dpc) of adhesion

[1] To whom correspondence should be addressed. Fax: +46 31 41 61 08. E-mail: henrik.semb@medkem.gu.se.

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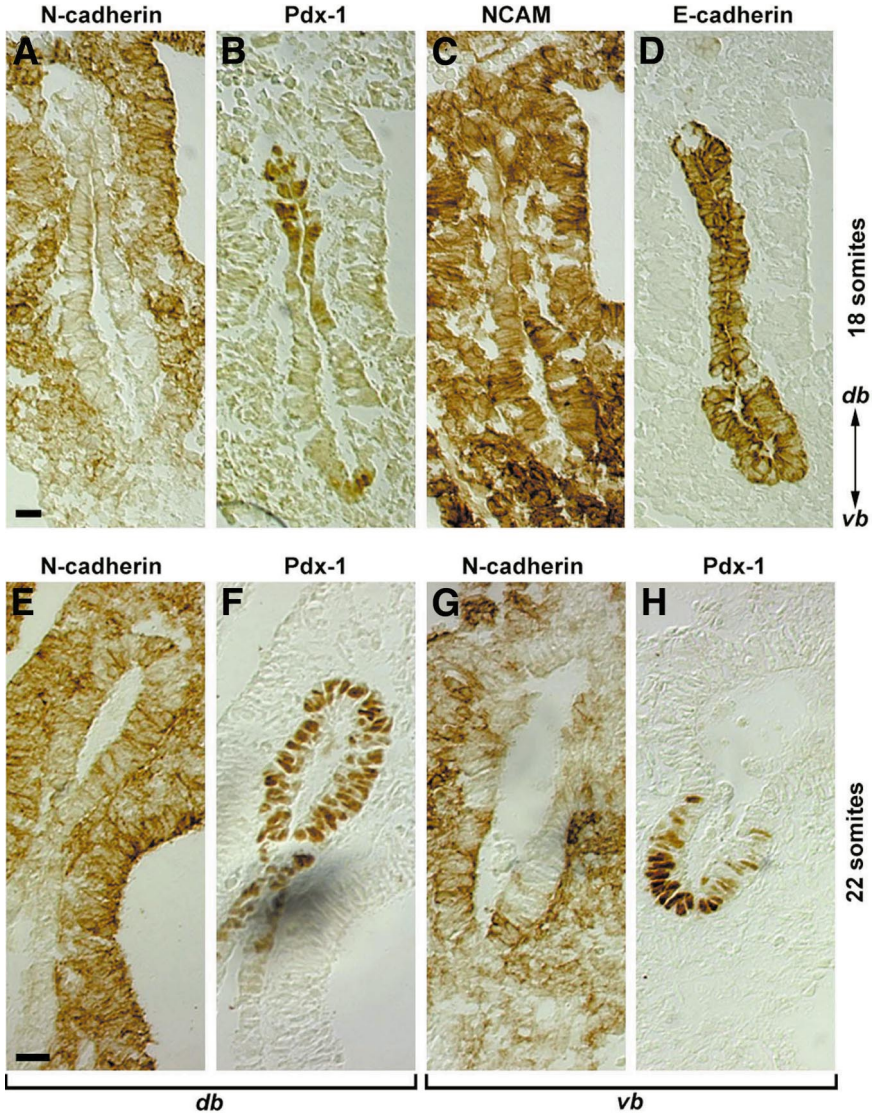


FIG. 1. Expression of N-cadherin during early stages of pancreas development. Immunohistochemical analysis of consecutive transverse sections of 18–20 somites (9.5 dpc) (A–D) and 22 somites (late 9.5 dpc) (E–H) pancreata using antibodies against N-cadherin (A, E, G), Pdx1 (B, F, H), N-CAM (C), and E-cadherin (D). Until the 22-somite stage N-cadherin was detected only in the mesenchyme surrounding the dorsal (db) and ventral (vb) pancreatic buds in a dorsal–ventral gradient, with the highest levels in the dorsolateral mesenchyme. At the 22-somite stage N-cadherin was expressed in both the pancreatic endoderm and surrounding mesenchyme. Scale bars = 20 μm.

defects during early heart development (Radice *et al.*, 1997). The fact that N-cadherin is expressed in mesenchymal cells, and occasionally in epithelial cells, during early phases of organogenesis suggests that N-cadherin may play an important role during epithelial–mesenchymal interactions as well.

Limited expression and functional analysis suggest that N-cadherin may be functionally involved in pancreatic morphogenesis (Dahl *et al.*, 1996; Moller *et al.*, 1992). The pancreas is derived from pancreatic progenitor cells forming a dorsal and ventral evagination of the foregut endoderm. During the outgrowth of these buds a branched ductular tree, containing pancreatic progenitors, is formed (Edlund, 1999; Pictet *et al.*, 1976; Slack, 1995). It is thought that the organization of the endocrine cells into islets of Langerhans occurs through a series of morphogenetic events involving cell migration and cell adhesion. During the initial stages of pancreatic development N-cadherin is expressed both in the mesenchymal and epithelial compartment. Here we show that mice

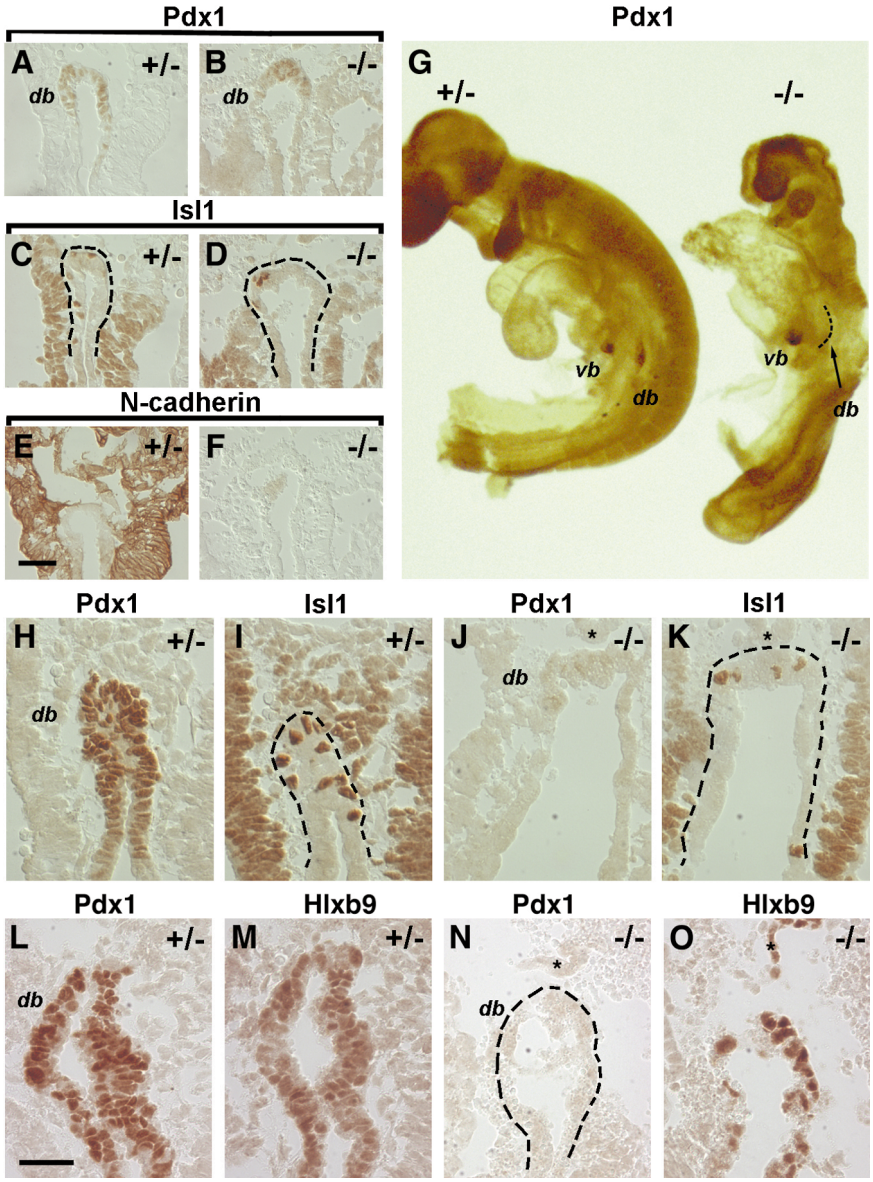


FIG. 2. N-cadherin-deficient embryos selectively lack a dorsal pancreas. (A, C, E) Immunohistochemical analysis of consecutive transverse sections of 9.0 dpc heterozygous embryo using antibodies against Pdx1 (A), Isl1 (C), and N-cadherin (E). (B, D, F) Immunohistochemical analysis of consecutive transverse sections of 9.0 dpc N-cadherin-deficient embryo using antibodies against Pdx1 (B), Isl1 (D), and N-cadherin (F). At 9.0 dpc Pdx1 was expressed at comparable levels in heterozygous and homozygous dorsal pancreatic endoderm. (G) Whole-mount immunohistochemistry on 9.5 dpc N-cadherin heterozygous (+/−) and homozygous (−/−) embryos using Pdx1 antibodies. At 9.5 dpc Pdx1 was expressed in both pancreatic buds in heterozygous embryos, whereas it was detected only in the ventral pancreatic endoderm in *N-cadherin* −/− embryos. The arrow and broken line indicate the absent dorsal bud. (H–K) Immunohistochemical analysis of consecutive transverse sections of 9.5 dpc heterozygous (H, I) and homozygous (J, K) embryos using antibodies against Pdx1 (H, J) and Isl1 (I, K). (L–O) Immunohistochemical analysis of consecutive transverse sections of 9.5 dpc heterozygous (L, M) and homozygous (N, O) embryos using antibodies against Pdx1 (L, N) and Hlxb9 (M, O). Asterisks indicate the notochord. In C, D, I, K, and N the border between the epithelium and mesenchyme is indicated by a broken line. db, dorsal buds; vb, ventral buds. Scale bars = 40 μm.

lacking N-cadherin selectively lack a dorsal pancreas, and that the underlying mechanism involves a crucial function of N-cadherin in survival of the dorsal pancreatic mesenchyme.

MATERIALS AND METHODS

Animals

The generation of *N-cadherin* −/− mice was described earlier (Radice *et al.*, 1997) and +/+, +/−, and −/− embryos were obtained from our local breeding colony of *N-cadherin* +/− mice. Pancreatic development was indistinguishable between wild type and heterozygous mice. Mice and embryos were genotyped as described (Radice *et al.*, 1997).

Immunohistochemistry and in Situ Hybridization

For immunohistochemistry, embryos were fixed and sectioned as previously described (Esni *et al.*, 1999). *In situ* hybridization using *Isl1, Sonic hedgehog,* and *cadherin-11* probes was carried out as described (Boström *et al.*, 1996).

Immunoreagents

Antibodies directed against E-cadherin, N-cadherin, N-CAM, Pdx1, glucagon, and insulin were used as described (Esni *et al.*, 1999). In addition, the following antibodies were used at the indicated dilutions: rabbit anti-Isl1 (1:250) (Ahlgren *et al.*, 1997), rabbit anti-Hlxb9 (1:8000) (Harrison *et al.*, 1999), and rabbit anti-human α-amylase (1:1000; Sigma). The following antibodies were purchased from Jackson ImmunoResearch Laboratories Inc. and diluted as indicated: biotin-conjugated anti-rat, anti-rabbit, anti-guinea pig, and FITC-conjugated anti-rabbit (1:500). Cy3-conjugated and FITC-conjugated strepavidin were used at 1:1000 and 1:500 dilutions, respectively. The Vectastain ABC kit was from Vector Laboratories Inc.

Whole-Mount Immunohistochemistry

Whole-mount immunohistochemistry was performed as described (Ohlsson *et al.*, 1993).

Culture of Pancreatic Rudiment

Isolation, recombination, and culture of pancreatic rudiments were carried out essentially as described by Gittes and Galante (1993) and Ahlgren *et al.* (1997). The mesenchyme was removed from the endoderm without using trypsin. The cultures were then maintained for 7 days in a humidified incubator at 37°C with 10% CO_2 with a change of medium every second day. The explants were then fixed in 4% paraformaldehyde in PBS (0.1 M PB + 0.15 M NaCl) at room temperature for 30 min, washed with PBS for 30 min, embedded in Tissue Tek compound, frozen on dry ice, and sectioned. Immunohistochemistry on sections was performed as above. Under these experimental conditions using endoderm from 18-somite stage embryos or older the same result was obtained in each experiment: two buds in heterozygous explants (n = 30), one bud in homozygous explants (n = 20), and two buds in the recombination experiments (n = 8).

Transmission Electronmicroscopy

Whole embryos were fixed overnight in 2.5% glutaraldehyde and 2% paraformaldehyde in 0.5 M sodium cacodylate buffer, pH 7.2. Postfixation was performed for 1 h with 0.5% OsO_4 and 1% potassium ferrocyanide in 0.5 M cacodylate at 4°C, and after rinsing in water the specimens were treated en bloc with 0.5% uranyl acetate. Dehydration in ethanol and propylene oxide was followed by epoxy resin (Agar 100) infiltration, whole-mount embedding, and curing by heat. Selected parts of embryos were serially sectioned on Reichert Ultracut E ultramicrotome fitted with a diamond knife. At every 10 μm, a 1 μm thick section was obtained for light microscopy. This was followed by ultrathin microtome advancement and the next 8–10 sections (≈50 nm) were collected on copper grids. The microtome was again set to 1 μm sections, and the next 9 sections were discarded. From each embryo about 30 sectioning cycles were performed. LM sections were stained with Richardson's stain and EM sections were contrasted with uranyl acetate and lead citrate before examination in a Zeiss CEM 902 electron microscope.

TUNEL Assay

The TUNEL assay was performed using terminal transferase (Boehringer) and biotin-16-dUTP (Boehringer). Sections were post-fixed in 4% paraformaldehyde/PBS for 15 min at room temperature, then washed three times for 5 min in sterile PBS, followed by incubation in 100 μl per slide prereaction mix (1× TdT buffer, 1.5 mM $CoCl_2$) for 10 min at room temperature. Sections were then incubated for 1 h at 37°C in a humidified chamber in 100 μl reaction mix (prereaction mix supplied with 50 U terminal transferase and 4 μM biotin-16-dUTP) covered by parafilm. Following three 5-min washes in PBS, and 1-h incubation at room temperature in 10% fetal calf serum, sections were visualized with FITC-conjugated streptavidin as described above. In the case of double immunofluorescence, the additional staining was performed as above following the TUNEL assay.

RESULTS

N-Cadherin Expression during Early Pancreatic Development

During the initial phases of pancreatic ontogeny (up to 20 somites, 9.5 dpc), N-cadherin was selectively expressed in the mesenchyme that surrounds both pancreatic anlagen and the gut (Fig. 1A). It is noteworthy that, in contrast to N-CAM's uniform expression pattern within the pancreatic mesenchyme (Fig. 1C), N-cadherin appeared to be transiently expressed in a dorsal–ventral gradient, with the highest levels in the dorsolateral and the lowest in the ventral mesenchyme (Fig. 1A). Its absence from the pancreatic endoderm was in contrast to E-cadherin, which was expressed in all epithelial cells of the pancreatic buds and gut (Fig. 1D). Subsequent to formation of the buds, N-cadherin appeared in the pancreatic endoderm (22 somites, late 9.5 dpc) (Figs. 1E and 1G).

During endocrine cell differentiation N-cadherin appeared concomitant with hormone synthesis and from 12.5

dpc and onward N-cadherin became restricted to aggregates of endocrine cells (data not shown).

Selective Dorsal Pancreas Agenesis in N-Cadherin-Deficient Mice

The observation that N-cadherin is expressed in pancreatic mesenchyme and endoderm and later in islet cells raised the question as to whether N-cadherin modulates mesenchymal–epithelial interactions as well as islet morphogenesis during pancreatic ontogeny. To investigate the functional role of N-cadherin in pancreatic organogenesis, we analyzed N-cadherin-deficient mice. However, because mice lacking N-cadherin die at around 10 dpc because of heart failure (Radice *et al.*, 1997), we could analyze only the initial stages of pancreatic development. Immunohistochemical analyses, using antibodies against Pdx1, a marker for the committed pancreatic epithelium, demonstrated that only the ventral pancreatic bud was detected in 9.5 dpc N-cadherin-deficient embryos (Figs. 2G, 2J, and 2N). Although the dorsal bud was absent, the dorsal pancreatic epithelium appeared to be committed based on the expression of Hlxb9 (Fig. 2O), the presence of Isl1-expressing endocrine precursor cells (Figs. 2D, 2K, 3D), and the lack of Sonic hedgehog expression (Fig. 3E). Whereas Hlxb9-deficient mice lack the dorsal pancreas (Harrison *et al.*, 1999; Li *et al.*, 1999), ectopic Sonic hedgehog expression within the pancreatic epithelium results in suppression of pancreatic development and enhancement of intestinal development (Apelqvist *et al.*, 1997). The functional role of Isl1 in pancreatic development is described below.

Pdx1 expression is first seen in the pancreatic endoderm at 8.5 dpc (10 somites), and mice lacking Pdx1 are born with small pancreatic rudiments (Jonsson *et al.*, 1994). To examine whether N-cadherin is required for the induction or maintenance of Pdx1 within the dorsal pancreatic endoderm, earlier stage embryos (9.0 dpc) were analyzed. Apparently, at this stage Pdx1 expression within the dorsal pancreatic endoderm in N-cadherin-deficient embryos was comparable to the expression observed in heterozygous embryos (Figs. 2A and 2B). Thus, N-cadherin is clearly necessary for the maintenance of Pdx1 within the dorsal pancreatic endoderm.

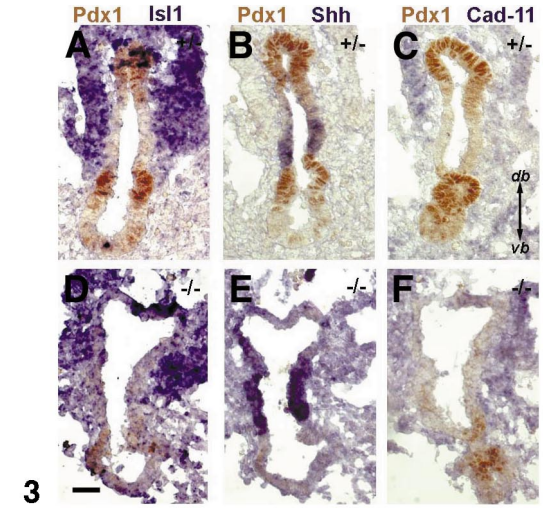
The fact that the ventral bud forms later than the dorsal bud (Spooner *et al.*, 1970) suggested that in the absence of N-cadherin either the dorsal bud did not form or its growth was severely delayed. To examine the latter possibility we cultured 9.5 dpc gut segments containing the pancreatic anlagen *in vitro*. Analyses of 7-day explant cultures showed that only growth and differentiation of one pancreas occurred in mutant explants, indicating that the lack of a dorsal bud in N-cadherin-deficient mice was attributable to agenesis (Figs. 6A and 6B).

Decreased Survival of Dorsal Pancreatic Mesenchyme

We were guided in the search for a possible mechanism for the selective lack of the dorsal pancreas in N-cadherin-deficient mice by the fact that histological evidence suggested that the dorsal pancreatic mesenchyme was absent in the mutant animals (Fig. 2J). To investigate this in greater detail we analyzed expression of Isl1 mRNA, a LIM-homeodomain transcription factor, which is expressed in the dorsal pancreatic and lateral gut mesenchyme and in presumptive endocrine cells within the endoderm (Ahlgren *et al.*, 1997). Although Isl1-positive cells were detected in the endoderm, the dorsal pancreatic mesenchymal Isl1-expressing cells were absent (Figs. 2K and 3E). Thus, taken together the data suggest that the lack of a dorsal pancreas is caused by a failure of mesenchyme-to-epithelium signaling as a result of the absence of dorsal pancreatic mesenchymal cells.

The dorsal pancreatic mesenchyme is most likely formed by the recruitment of mesenchymal cells in close vicinity (e.g., from splanchnic mesenchyme). To begin to resolve the mechanism for the lack of dorsal pancreatic mesenchyme in N-cadherin-deficient mice, we first examined mesenchymal cell apoptosis by employing TUNEL assays. Whereas few apoptotic cells were found in heterozygous mice, a significant fraction of apoptotic mesenchymal cells were identified closely associated with the presumptive dorsal pancreatic endoderm in homozygous mice (Figs. 4, 5E, and 5F). This was in contrast to the ventral pancreatic mesenchyme, which displayed no increased rate of apoptosis (Fig. 4B). Moreover, no significant change in pancreatic endoderm apoptosis was observed (Figs. 4 and 5).

To examine the consequence of decreased survival of mesenchymal cells on the dorsal pancreatic endoderm, we turned to ultrastructural analysis. In heterozygous embryos the dorsal pancreatic endoderm was arranged as a multicellular ridge with smoothly concave sides (Fig. 5A). Along a longitudinal distance of $\approx$20 μm at the summit of the ridge the very few cells in a median position exhibited numerous long and slender cytoplasmic extensions radiating in a dorsal and dorsolateral direction (Figs. 5A and 5C). These filipodia-like structures contained bundles of microfilaments and decorated a restricted part of the cells that was also characterized by a focal subplasmalemmal condensation of microfilaments oriented parallel with the cell surface (Figs. 5A and 5C; asterisks in 5C). A periendodermal basal lamina followed closely the external aspect of the lateral sides of the pancreatic anlage but continued as two stretched sheets along the median plane in a dorsal direction, separated by a distance of about one cell diameter. The tips of the filipodia of the median cells made several intimate contacts with the medial aspect of this basal lamina sleeve without penetrating it (arrows in Fig. 5C). Similar cytoplasmic extensions were not observed in the neighboring gut endoderm (data not shown). The



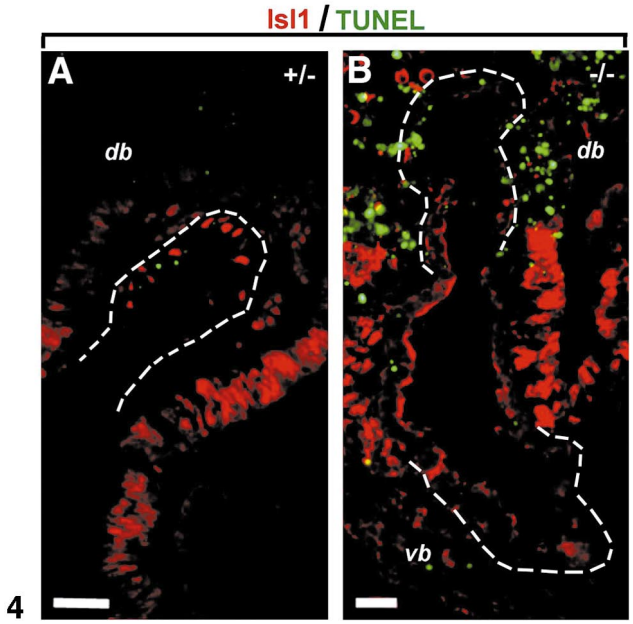


FIG. 3. Expression of Isl1, Sonic hedgehog, and cadherin-11 is unaffected in pancreatic anlagen of N-cadherin-deficient embryos. (A–C) Double-label immunohistochemistry and *in situ* hybridization on transverse sections of *N-cadherin* +/− 9.5 dpc pancreas using Pdx1 antibodies (brown) and *Isl1* (A), *Sonic hedgehog* (B), and *cadherin-11* (C) anti-sense mRNA probes (dark blue). (D–F) Double-label immunohistochemistry and *in situ* hybridization on transverse sections of *N-cadherin* −/− 9.5 dpc pancreas using Pdx1 antibodies (brown) and *Isl1* (D), *Sonic hedgehog* (E), and *cadherin-11* (F) anti-sense mRNA probes (dark blue). Compared to that in heterozygous embryos *Isl1* mRNA was unaffected within the lateral gut mesenchyme and dorsal pancreatic endoderm. Furthermore, *Sonic hedgehog* and *cadherin-11* mRNAs were unaffected within the endoderm and mesenchyme, respectively. db, dorsal buds; vb, ventral buds. Scale bar = 20 μm.

FIG. 4. Dorsolateral N-cadherin null pancreatic mesenchyme undergoes apoptosis. (A, B) TUNEL assay (green) on transverse sections of *N-cadherin* +/− (A) and *N-cadherin* −/− (B) 9.5 dpc pancreas followed by immunofluorescence analysis using Isl1 antibodies (red). In *N-cadherin* −/− embryos (B) extensive apoptosis was observed in the dorsolateral mesenchyme. db, dorsal buds; vb, ventral buds. Broken lines indicate pancreatic endoderm. Scale bars = 20 μm.

N-cadherin −/− pancreatic endoderm did not form a multicellular ridge and no signs of filipodia formation or cytoskeletal reorganization were observed in cells close to the median plane (Figs. 5B and 5D). Although, as in the heterozygotes, the basal lamina of the epithelium did not form a complete cover over the dorsal midplane (arrowheads in Fig. 5D), it ended on both sides as curled loose ends reaching only a few micrometers away the endoderm (Fig. 5D). The differences revealed in the electron microscope have an obvious bearing on the local regulation of cell shape and motility and should contribute to explain the absence of a dorsal bud in N-cadherin null embryos. The organization of cell–cell junctions, including tight junctions and adherens junctions, appeared unaffected within the *N-cadherin* −/− endoderm (Fig. 5). The ultrastructural analysis also corroborated our earlier observation of an increased number of apoptotic mesenchymal cells closely associated with the presumptive dorsal pancreatic endoderm (Fig. 5F).

N-Cadherin Is Not Required within the Pancreatic Endoderm

The fact that N-cadherin was expressed in the mesenchyme, but not in the endoderm, during the initial stages of dorsal bud formation suggests a specific role for N-cadherin in the survival of dorsal pancreatic mesenchyme. However, to exclude the possibility that N-cadherin also has a function in the pancreatic endoderm, we performed recombination experiments with mutant pancreatic gut explants and wild type dorsal pancreatic mesenchyme. These experiments demonstrated that, in the presence of wild type mesenchyme, *N-cadherin* −/− dorsal endoderm underwent evagination, branching morphogenesis, and exocrine and endocrine cell type cytodifferentiation (Fig. 6). These data demonstrate that the requirement of N-cadherin during formation of the dorsal pancreas is within the mesenchyme and not within the endoderm.

Initial Endocrine Cell Differentiation and Clustering Occur in the Absence of N-Cadherin

The observations that N-cadherin protein was first seen concomitant with hormone expression and that hormone-producing cells were never detected in the mutant dorsal endoderm (data not shown), implied a potential role for N-cadherin in endocrine cell differentiation. However, the

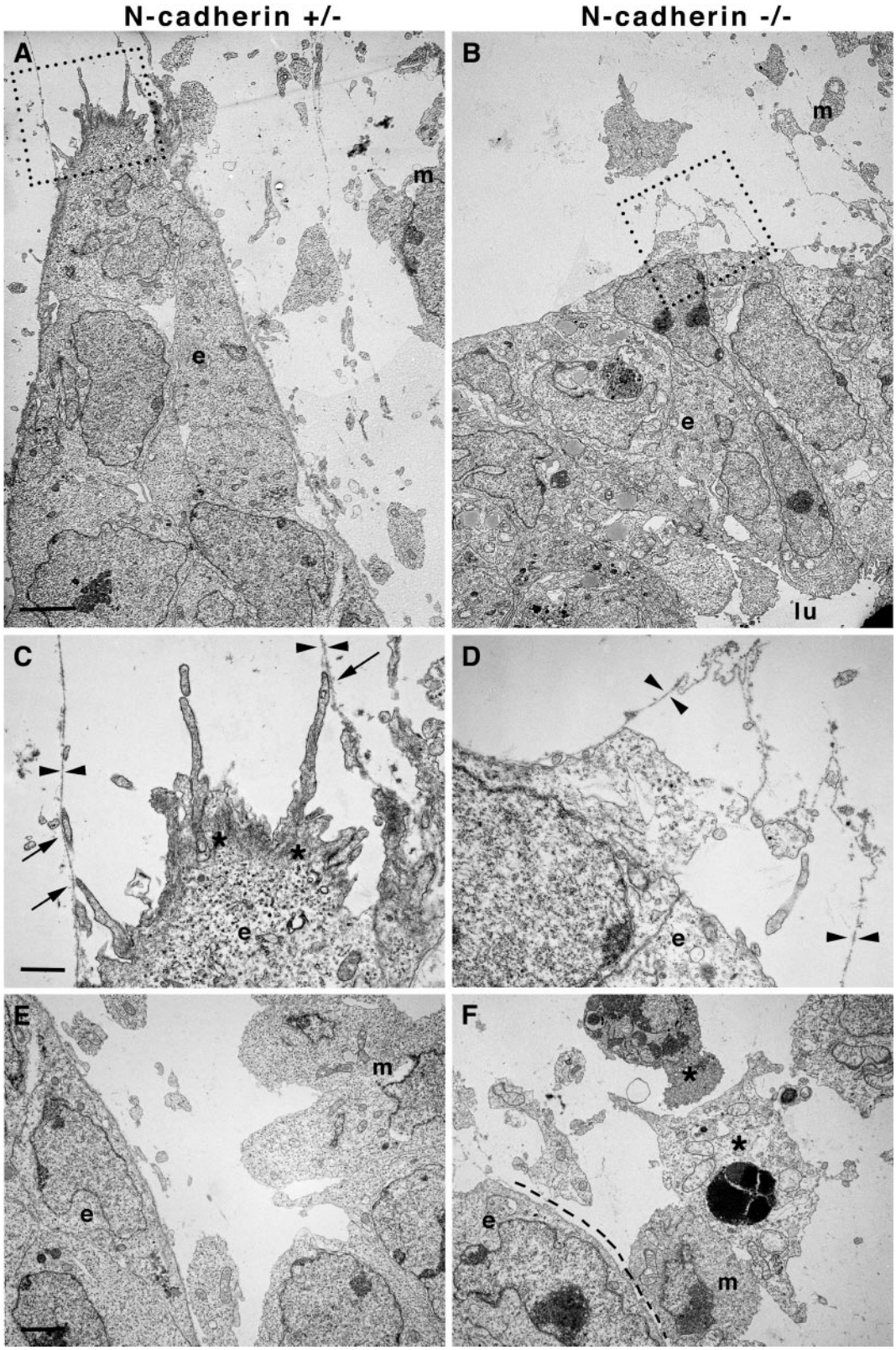


FIG. 5. N-cadherin-deficient mice exhibit ultrastructural alterations in the dorsal pancreatic endoderm. (A, B) Electron photomicrographs of 9.5 dpc dorsal pancreatic buds from heterozygous (A, +/−) and homozygous (B, −/−) animals. Scale bar = 5 μm. (C, D) The rectangular areas indicated in A and B are shown at higher magnifications in C and D, respectively. In heterozygous endoderm evidence of cytoskeletal condensations (asterisks, in C) associated with cellular extensions, or filipodia, extending dorsally and making contacts (arrows) with the basal lamina (arrowheads) were observed, whereas in homozygous endoderm neither filipodia nor cytoskeletal aggregates were detected (D). Scale bar = 1 μm. (E, F) Although apoptotic cells were scarce in heterozygous pancreatic dorsal mesenchyme (E), apoptotic mesenchymal cells were frequently observed associated with *N-cadherin* −/− dorsal pancreatic endoderm (asterisks in F). In F the border between endoderm and mesenchyme is indicated by a broken line. Scale bar = 2.5 μm. Dorsal pancreatic endoderm, dorsal pancreatic mesenchyme, and gut lumen are indicated by e, m, and lu, respectively.

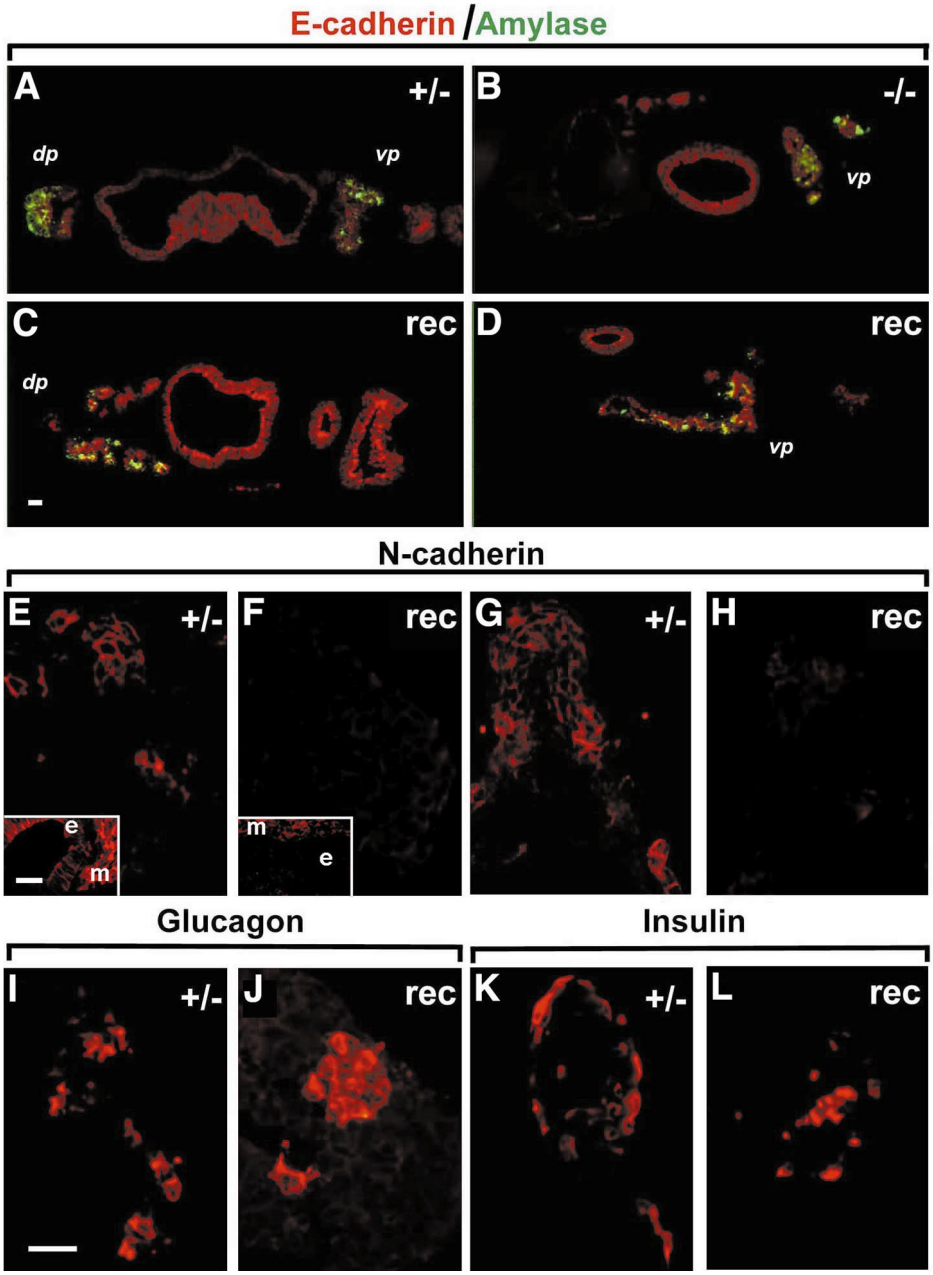


FIG. 6. Mutant endoderm forms a pancreas in the presence of wild type mesenchyme. (A–D) Double immunofluorescence stainings on sections of 7-day-cultured pancreatic explants using amylase (green) and E-cadherin (red) antibodies. In heterozygous 9.5 dpc explant cultures both the dorsal (dp) and the ventral (vp) pancreas formed (A), whereas the ventral pancreas (vp) selectively developed in 9.5 dpc N-cadherin-deficient explants (B). Wild type 10.5 dpc mesenchyme cocultured with 9.5 dpc mutant endoderm (rec) induced formation of both the dorsal (dp, C) and ventral (vp, D) pancreas. In C and D the dorsal and ventral pancreas are shown on sections from different regions of the same explant. (E–L) Immunofluorescence stainings on consecutive sections of 9.5 dpc explants derived from heterozygous embryos (E, G, I, K) and 9.5 dpc mutant endoderm cocultured with 10.5 dpc wild type mesenchyme (F, H, J, L) using N-cadherin (E–H), glucagon (I, J), and insulin (K, L) antibodies. These results indicate that N-cadherin is not necessary for the initial differentiation and clustering of α and β cells. Moreover, the lack of N-cadherin expression in α cells (F, J) and β cells (H, L) derived from reconstitution experiments demonstrates that endocrine cells are derived from N-cadherin-deficient pancreatic endoderm. Inset in E shows N-cadherin expression in heterozygous gut epithelium (e) and mesenchyme (m), whereas inset in F demonstrates the absence and presence of N-cadherin in homozygous gut epithelium (e) and wild-type mesenchyme (m), respectively. Scale bars = 20 μm.

initial endocrine cell commitment appeared normal, given that Isl1-expressing cells were identified in mutant endoderm (Figs. 2D and 2K). By examining pancreatic gut explants grown *in vitro,* we could determine whether endocrine cytodifferentiation was affected in the absence of N-cadherin. Given the early stages of gut explant isolation only α and β cells could be identified in wild type explants after 7 days of culture. Because clusters of α (Figs. 6I and 6J) and β (Figs. 6K and 6L) cells were found in both heterozygous and N-cadherin-deficient explants recombined with wild type mesenchyme, we conclude that neither the initial differentiation nor clustering of endocrine cells requires N-cadherin.

DISCUSSION

By analyzing the initial stages of pancreatic development in N-cadherin-deficient mice, we could for the first time identify a factor that is required for the survival of the dorsal pancreatic mesenchyme. Consequently, in mice homozygous for a null mutation of N-cadherin no dorsal pancreas forms. Regarding the early differentiation and clustering of endocrine cells N-cadherin appears to be dispensable. The finding that no pancreas forms in the absence of the surrounding mesenchyme is in agreement with earlier studies of the requirement of the mesenchyme for growth and differentiation of the pancreas (Edlund, 1999). However, so far, no data supporting a crucial role for cadherin-mediated cell–cell interactions in mesenchymal cell survival during organogenesis have been reported.

It is well established that the pancreatic mesenchyme is necessary for growth and differentiation of the pancreatic endoderm (Edlund, 1999). This growth and differentiation have been defined as cell proliferation and differentiation into exocrine and endocrine pancreatic cell types, respectively. However, how the mesenchyme affects epithelial cell behavior in the pancreatic endoderm to form an evagination has not yet been resolved. Here, we present results suggesting that signals from the mesenchyme may promote rearrangement of the cytoskeletal filament system, presumably filamentous actin, within the endoderm. Furthermore, these signals appear to guide the formation of dorsally oriented cytoplasmic extensions, or filipodia. The frequent observation of connections between the tips of filipodia and the dorsally extending basal lamina suggest that the basal lamina may guide these cellular extensions. Thus, that not only growth but also cell shape changes, including formation of cytoplasmic extensions, are influenced by the dorsal mesenchyme, indicate that the mesenchyme regulates several aspects of early pancreatic morphogenesis. The absence of cytoskeletal condensations and filipodia in *N-cadherin* $-/-$ dorsal pancreatic endoderm gives mechanistic insight into how N-cadherin and the dorsal mesenchyme may affect pancreatic epithelial cell behavior to drive the formation of a dorsal bud.

The finding that the dorsal pancreas is selectively affected in N-cadherin null animals suggests differences in the mechanisms for morphogenesis of the ventral and dorsal pancreas, respectively. Indeed, the major difference between the affected dorsal pancreatic mesenchyme and the unaffected lateral gut and ventral pancreatic mesenchyme is that the dorsal mesenchyme is recruited, whereas the lateral and ventral mesenchyme is splanchnic derived and present before gut closure and pancreas commitment. Consequently, the different mechanisms for formation of the ventral and dorsal pancreatic mesenchyme, respectively, together with N-cadherin's selective role in dorsal mesenchyme formation, suggest that N-cadherin may play a specific role in the recruitment of the dorsal pancreatic mesenchyme. In such a speculative scenario ablation of N-cadherin could thus result in perturbed mesenchymal cell recruitment and apoptosis. In support of the suggested role of N-cadherin in the recruitment of mesenchymal cells are previous findings that N-cadherin plays an active role in cell migration (Hazan *et al.,* 2000; Matsunaga *et al.,* 1988; Nieman *et al.,* 1999). Alternatively, other CAMs expressed in the mesenchyme, such as cadherin-11 (Figs. 3C and 3F) and N-CAM (Fig. 1C) may compensate for the lack of N-cadherin in the lateral and ventral mesenchyme.

Recently, it was demonstrated that Isl1 is required for formation of the dorsal pancreatic mesenchyme (Ahlgren *et al.,* 1997). Thus, the absence of the dorsal pancreatic mesenchyme in *N-cadherin* $-/-$ mice is reminiscent of the phenotype of Isl1-deficient mice. To examine whether a possible molecular link exists between N-cadherin and Isl1 in the developing pancreas we investigated Isl1 expression in N-cadherin mutant mice. Obviously we could not perform these studies in the dorsal pancreatic mesenchyme because it is lacking. However, within the lateral gut mesenchyme, which coexpresses both molecules, Isl1 expression was unaffected in *N-cadherin* $-/-$ mice, suggesting that at least in the lateral mesenchyme Isl1 is not molecularly linked downstream to N-cadherin.

We previously demonstrated that when the function of all classic cadherins expressed in pancreatic β cells, including E-cadherin, N-cadherin, and R-cadherin (Begemann *et al.,* 1990; Dahl *et al.,* 1996; Hutton *et al.,* 1993; Moller *et al.,* 1992; Rouiller *et al.,* 1991) is inhibited, the initial aggregation of β cells is severely affected (Dahl *et al.,* 1996). Gene-targeted inactivation of either N-cadherin or R-cadherin (U. Dahl and H. Semb, unpublished observations) has no apparent effect on the initial clustering of endocrine cells, demonstrating that neither of these cadherins is essential for endocrine cell clustering. Consequently, either E-cadherin is the main regulator of aggregation of pancreatic endocrine cells or functional redundancy exists between cadherins in terms of cell clustering.

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